10-16-99 Rec'd PCT/PTO 13 OCT 2000 PCT 8

FORM PTO-1390 (REV 10-95) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 9320.113USWO

U S APPLICATION NO. (If known, see 37 C F R 1 5): Unknown 09/673555

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/00915	April 19, 1999	April 20, 1998

TITLE OF INVENTION

AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USES

APPLICANT(S) FOR DO/EO/US

BENVENISTE et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An unsigned oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND of SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: Preliminary Amendment; International Preliminary Examination Report; International Search Report; 2 cited references

U.S. APPLICATION NO (If known, see 37 C F R. 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Unknown 09/673555	PCT/FR99/00915	9320.113USWO

17. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a) (1)-(5)):		
Search Report has been prepared by the EPO or JPO **$860.00**		
International preliminary examination fee paid to U.S. Patent and Trademark Office (37 CFR 1.492(a)(1)) **$690.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$710.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(3)) paid to USPTO **$1000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	**$0**	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total claims	72	-20 =	52	X **$18.00**	**$936.00**	
Independent claims	5	-3 =	2	X **$80.00**	**$160.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+ **$270.00**	**$0**	
TOTAL OF ABOVE CALCULATIONS =					**$1956.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					**$0**	
SUBTOTAL =					**$1956.00**	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f).				+	**$0**	
TOTAL NATIONAL FEE =					**$1956.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property				+	**$0**	
TOTAL FEES ENCLOSED =					**$1956.00**	
					Amount to be: refunded	**$0**
					charged	**$0**

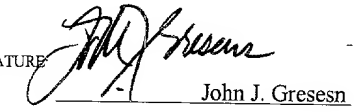
a. [X] Check(s) in the amount of $1956.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-2725.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
John J. Gresens
MERCHANT & GOULD
P.O. Box 2903
Minneapolis, MN 55402-0903

SIGNATURE 

John J. Gresesn
NAME

33,112
REGISTRATION NUMBER

526 Rec'd PCT/PTO 04 DEC 2000

09/673131

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	BENVENISTE et al.	Examiner:	Unknown
Serial No.:	09/673,555	Group Art Unit:	Unknown
Filed:	October 13, 2000	Docket:	9320.113USWO
Notice of Allow. Date:	n/a	Batch No.:	Unknown
Due Date:	December 7, 2000		

Title: AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USE

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this Transmittal Letter and the paper, as described herein, are being deposited in the United States Postal Service, as first class mail, with sufficient postage, in an envelope addressed to: BOX MISSING REQUIREMENTS Assistant Commissioner for Patents, Washington, D.C. 20231, on November 29, 2000

By: Todd Michel

OIPE JC38 DEC 04 2000 PATENT & TRADEMARK OFFICE

BOX MISSING REQUIREMENTS
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We are transmitting herewith the attached:

- ☒ Transmittal Sheet in duplicate containing Certificate of Mailing
- ☒ Signed Combined Declaration and Power of Attorney
- ☒ Check in the amount of $130.00 for missing requirements fee
- ☒ Other: Notification of Missing Requirements form
- ☒ Return postcard

Please consider this a PETITION FOR EXTENSION OF TIME for a sufficient number of months to enter these papers, if appropriate. Please charge any additional fees or credit overpayment to Deposit Account No. 13-2725. A duplicate of this sheet is enclosed.

MERCHANT & GOULD P.C.
P.O. Box 2903, Minneapolis, MN 55402-0903
612.332.5300

23552
PATENT TRADEMARK OFFICE

By: John J. Gresens
Name: John J. Gresens
Reg. No.: 33,112
JJG/tvm

12/07/2000 ERIMANDO 00000018 09673555
01 FC:154 130.00 OP

S/N Unknown

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	BENVENISTE et al.	Examiner:	Unknown
Serial No.:	Unknown	Group Art Unit:	Unknown
Filed:	October 13, 2000	Docket No.:	9320.113USWO
Title:	AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USE		

CERTIFICATE UNDER 37 CFR 1.10
'Express Mail' mailing label number: EL649974803US
Date of Deposit: October 13, 2000
I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C . 20231.

By: Linda McCormick
Name: Linda McCormick

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D. C. 20231

Dear Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment, which is based on the Article 34.2 amendments, based on claims amended in prosecution of the international application and published in the International Preliminary Examination Report, a copy of which is enclosed herewith:

IN THE SPECIFICATION

A courtesy copy of the present specification is enclosed herewith. However, the World Intellectual Property Office (WIPO) copy should be relied upon if it is already in the U.S. Patent Office.

IN THE CLAIMS

In claim 6, lines 1 & 2, delete "or claim 5"

In claim 7, lines 1 & 2, delete "any one of claims 2 to 6" and insert--claim 2--

In claim 8, lines 1 & 2, delete "any one of claims 2 to 6" and insert--claim 2--

In claim 11, lines 1 & 2, delete "or claim 10"

In claim 13, lines 1 & 2, delete "any one of claims 9 to 12" and insert--claim 9--

In claim 14, lines 1 & 2, delete "any one of claims 10 to 12" and insert--claim 10--

In claim 15, lines 1 & 2, delete "any one of claims 1 to 14" and insert--claim 1--

In claim 17, lines 1 & 2, delete "any one of claims 1 to 16" and insert--claim 1--

In claim 18, lines 1 & 2, delete "any one of claims 1 to 17" and insert--claim 9--

In claim 20, lines 5 & 6, delete "any one of the claims 1 to 7 and 9 to 19" and insert --claim 1--

In claim 26, line 1, delete "or claim 25"

In claim 27, line 1, delete "any one of claims 24 to 26" and insert--claim 24--

In claim 28, line 3, delete "any one of claims 20 to 23" and insert--claim 20--

In claim 29, line 3, delete "any one of claims 20 to 23" and insert--claim 20--

In claim 30, lines 6 & 7, delete "any one of the claims 1 to 8 and 15 to 19" and insert --claim 1--

In claim 34, line 1, delete "any one of claims 32 or 33" and insert--claim 32--

In claim 35, line 1, delete "any one of claims 32 to 34" and insert--claim 32--

In claim 38, lines 1 & 2, delete "any one of the claims 36 or 37" and insert--claim 36--

In claim 41, line 1, delete "any one of claims 39 or 40" and insert--claim 39--

In claim 47, lines 1 & 2, delete "or claim 46"

In claim 48, lines 1 & 2, delete "any one of the claims 43 to 47" and insert--claim 43-

In claim 49, lines 1 & 2, delete "any one of claims 43 to 47" and insert--claim 43-

In claim 52, lines 1 & 2, delete "or claim 51"

In claim 54, lines 1 & 2, delete "any one of claims 50 to 53" and insert--claim 50--

In claim 55, lines 1 & 2, delete "any one of claims 51 to 53" and insert--claim 51--

In claim 56, lines 1 & 2, delete "any one of claims 42 to 55" and insert--claim 42--

In claim 58, lines 1 & 2, delete "any one of claims 42 to 57" and insert--claim 42--

In claim 59, lines 1 & 2, delete "any one of claims 42 to 58" and insert--claim 42--

In claim 61, lines 5 & 6, delete "any one of the claims 42 to 49 and 50 to 60" and insert --claim 42--

In claim 66, line 4, delete "claim 261" and insert--claim 61--

In claim 67, line 1, delete "or claim 66"

In claim 68, line 1, delete "any one of claims 65 to 67" and insert--claim 65--

In claim 69, line 3, delete "any one of the claims 60 to 64" and insert--claim 60--

In claim 70, line 3, delete "any one of claims 61 to 64" and insert--claim 61--

In claim 71, lines 5 & 6, delete "any one of the claims 42 to 49 and 56 to 60" and insert --claim 42--

REMARKS

The above preliminary amendment is made to remove multiple dependencies from claims 6, 7, 8, 11, 13, 14, 15, 17, 18, 20, 26, 27, 28, 29, 30, 34, 35, 38, 41, 47, 48, 49, 52, 54, 55, 56, 58, 59, 61, 66, 67, 68, 69, 70 and 71.

Applicants respectfully request that the preliminary amendment described herein be entered into the record prior to calculation of the filing fee and prior to examination and consideration of the above-identified application.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicants' primary attorney-of record, John J. Gresens (Reg. No. 33,112), at (612) 371.5265.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: October 13, 2000

By _____
John J. Gresens
Reg. No. 33,112

JJG/tvm

S/N 09/673,555

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	BENVENISTE et al.	Examiner:	Unknown
Serial No.:	09/673,555	Group Art Unit:	Unknown
Filed:	October 13, 2000	Docket No.:	9320.113USWO

Title: AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USE

CERTIFICATE UNDER 37 CFR 1.10
'Express Mail' mailing label number: EL650063217US
Date of Deposit: February 6, 2001
I hereby certify that this correspondence is being deposited with the United States Postal Service 'Express Mail Post Office To Addressee' service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C . 20231.

By: _____
Name: Brian Maharaj

PRELIMINARY AMENDMENT

BOX DEFECTIVE RESPONSE
Assistant Commissioner for Patents
Washington, D. C. 20231

Dear Sir:

In connection with the above-identified application filed herewith, please enter the following preliminary amendment, which is based on the Article 34.2 amendments, based on claims amended in prosecution of the international application and published in the International Preliminary Examination Report, a copy of which is enclosed herewith:

IN THE SPECIFICATION

A courtesy copy of the present specification is enclosed herewith. However, the World Intellectual Property Office (WIPO) copy should be relied upon if it is already in the U.S. Patent Office.

IN THE CLAIMS

Please amend the following claims:

In claim 6, lines 1 & 2, delete "or claim 5"

In claim 7, lines 1 & 2, delete "any one of claims 2 to 6" and insert--claim 2--

In claim 8, lines 1 & 2, delete "any one of claims 2 to 6" and insert--claim 2--

In claim 11, lines 1 & 2, delete "or claim 10"

In claim 13, lines 1 & 2, delete "any one of claims 9 to 12" and insert--claim 9--

In claim 14, lines 1 & 2, delete "any one of claims 10 to 12" and insert--claim 10--

In claim 15, lines 1 & 2, delete "any one of claims 1 to 14" and insert--claim 1--

In claim 17, lines 1 & 2, delete "any one of claims 1 to 16" and insert--claim 1--

In claim 18, lines 1 & 2, delete "any one of claims 1 to 17" and insert--claim 9--

In claim 20, lines 5 & 6, delete "any one of the claims 1 to 7 and 9 to 19" and insert --claim 1--

In claim 26, line 1, delete "or claim 25"

In claim 27, line 1, delete "any one of claims 24 to 26" and insert--claim 24--

In claim 28, line 3, delete "any one of claims 20 to 23" and insert--claim 20--

In claim 29, line 3, delete "any one of claims 20 to 23" and insert--claim 20--

In claim 30, lines 6 & 7, delete "any one of the claims 1 to 8 and 15 to 19" and insert --claim 1--

Please add the following new claims:

32. (New) A process for producing or acquiring from a substance (1) signals, particularly electrical signals, characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or of an active element contained in said substance;

said process including the stages:

- of placing said substance in a zone (13) subjected to an excitation field of an electrical, magnetic and/or electromagnetic type (15, 17); said excitation field being produced by an excitation signal having particularly a frequency between 20 Hz and 20 000 Hz;

- of converting the fields resulting from the interaction of the excitation field and the substance, into signals, particularly electrical signals, by means of a first transducer or acquisition sensor (5) receiving said resulting fields,

(said signals are characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance).

33. (New) A process according to claim 32, the characteristic of said excitation signal being that it has a uniform spectral power, of the white noise type.

34. (New) A process according to any one of claims 32, such that:

- the zone subjected to the excitation field is isolated (13) from the parasitic fields coming from the environment.

35. (New) A process according to any one of claims 32, further including the stage:

- of applying said signals coming from said first transducer (5), by means of a second transducer (51), to a biological receptor system,

(in such a way that the biological and/or chemical activity or the biological and/or chemical behaviour of the biological receptor system will be modified in accordance with the nature of the biological and/or chemical activity or the biological and/or chemical behaviour of said substance).

36. (New) A system for producing or acquiring signals, particularly electrical signals, characteristic of the biological and/or chemical activity or of the biological and/or chemical

behaviour of a substance (1) or of an active element contained in said substance and a system for implementing the properties of such signals;

said system including:

- an emitter (15, 17) generating an excitation field of an electrical, magnetic and/or electromagnetic type in a zone (13) where said substance is located; said emitter being excited by an excitation signal having particularly a frequency between 20 Hz and 20 000 Hz;

- a first transducer or acquisition sensor (5) receiving fields resulting from the interaction of said excitation field and said substance, said first transducer converting said resulting fields into signals, particularly electrical signals,

(said signals are characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance).

- emission means particularly a coil (51) for applying said signals coming from said first transducer to a biological receptor system,

(in such a way that the biological and/or chemical activity or the biological and/or chemical behaviour of the biological receptor system will be modified in accordance with the nature of the biological and/or chemical activity or the biological and/or chemical behaviour of said substance).

37. (New) A system according to claim 36, the characteristic of said excitation signal being that it has a uniform spectral power.

38. (New) A system according to any one of the claims 36, such that it further comprises:

- shielding means (13) to isolate said zone from the parasitic fields coming from the environment.

39. (New) A device for producing or acquiring signals, particularly electrical signals, characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of a substance or of an active element contained in said substance;

said device including:

- an emitter (15, 17) generating an excitation field of an electrical, magnetic and/or electromagnetic type in a zone (13) where said substance is located; said emitter being excited by an excitation signal having particularly a frequency between 20 Hz and 20 000 Hz;

- a first transducer or acquisition sensor (5) receiving fields resulting from the interaction of said excitation field and said substance, said first transducer converting said resulting fields into signals, particularly electrical signals,

(said signals are characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance).

40. (New) A device according to claim 39, the characteristic of said excitation signal being that it has a uniform spectral power.

41. (New) A device according to claim 39, such that it further comprises:

- shielding means (13) to isolate said zone from the parasitic fields coming from the environment.

42. (New) An amplification process of a reaction between the two elements of a ligand-receptor pair, characterised in that it includes:

- bringing into contact the two elements of a ligand-receptor pair in conditions suitable to allow their reaction, and

- previously, simultaneously or subsequently to this bringing into contact, the application to one and/or the other of these elements of an electromagnetic signal, obtained from an electrical signal

produced by a sensor placed in front of one and/or the other of the two elements of the ligand-receptor pair; said electromagnetic signal being hereinafter designated the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of a ligand-receptor pair.

43. (New) An amplification process according to claim 42, characterised in that the reaction between the ligand and the receptor is obtained by bringing into contact two reagents containing respectively the ligand and the receptor, and, to one and/or the other of these reagents, is applied an electromagnetic test signal suspected to include the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of a ligand-receptor pair.

44. (New) An amplification process according to claim 43, characterised in that the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by exposure of a solution or a suspension containing one or other of these reagents to this electromagnetic signal.

45. (New) An amplification process according to claim 43, characterised in that the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by dilution of a solution or a suspension including one and/or the other of these reagents, in a solvent having been previously exposed to this electromagnetic signal.

46. (New) An amplification process according to claim 43, characterised in that the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by dissolution or putting into suspension of this reagent or these reagents in a solvent having been previously exposed to this electromagnetic signal.

47. (New) An amplification process according to claim 45, characterised in that the solvent having been previously exposed to the electromagnetic test signal is water or physiological solute.

48. (New) An amplification process according to claim 43, characterised in that the electromagnetic test signal is the electromagnetic signal obtained from an electrical signal produced by a sensor placed in front of an analysis sample suspected to contain the ligand and/or the receptor.

49. (New) An amplification process according to claim 43, characterised in that the electromagnetic test signal is the electromagnetic signal radiated by an electromagnetic radiation source.

50. (New) An amplification process according to claim 42, characterised in that the reaction between the ligand and the receptor is made by bringing into contact an analysis sample suspected to contain the ligand and/or the receptor, with a reagent containing either the receptor, or the ligand, and, to this sample and/or to this reagent, is applied the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair.

51. (New) An amplification process according to claim 50, characterised in that the application, to the analysis sample, of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair is made by exposure of this sample to this electromagnetic signal or signals, or by dilution of this sample in a solvent having been previously exposed to said electromagnetic signal or signals.

52. (New) An amplification process according to claim 50, characterised in that the application, to the reagent intended to react with the analysis sample, of the electromagnetic

signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair is made by exposure of a solution or a suspension containing this reagent to this electromagnetic signal or signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to this electromagnetic signal or signals, or again by dissolution or putting into suspension of this reagent in a solvent having been previously exposed to said electromagnetic signal or signals.

53. (New) An amplification process according to claim 50, characterised in that, to the analysis sample and to the reagent intended to react with it, is applied the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair, by exposure of a solution or a suspension containing this sample and this reagent to this electromagnetic signal or signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to said electromagnetic signal or signals.

54. (New) An amplification process according to claim 50, characterised in that, to the analysis sample and/or to the reagent intended to react with it, is applied both said electromagnetic signal characteristic of the biological activity of the ligand and said electromagnetic signal characteristic of the biological activity of the receptor.

55. (New) An amplification process according to claim 51, characterised in that the solvent having been previously exposed to the electromagnetic signal or signals is advantageously water or physiological solute.

56. (New) An amplification process according to claim 42, characterised in that it includes an acquisition stage of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair.

57. (New) An amplification process according to claim 56, characterised in that it includes a recording and restitution stage of information representative of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair.

58. (New) An amplification process according to claim 42, characterised in that it includes a detection and, possibly, a measurement stage of the complexes resulting from the reaction between the ligand and the receptor.

59. (New) An amplification process according to claim 42, characterised in that the ligand is an antigen or a hapten, whereas the receptor is an antibody or a membranous receptor targeted specifically against this ligand.

60. (New) An amplification process according to claim 59, characterised in that the reaction between the antigen and the antibody or the hapten and the antibody is revealed by agglutination.

61. (New) A process for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it includes the implementation of an amplification process according to claim 42.

62. (New) A detection process according to claim 61, characterised in that it includes:

- the bringing into contact of two reagents containing respectively the ligand and the receptor, in conditions suitable to allow their reaction,
- previously, simultaneously or subsequently to this bringing into contact, the application, to one and/or the other of these reagents, of an electromagnetic signal obtained from an electrical signal produced by a sensor placed in front of the analytical sample; said electromagnetic signal being

hereinafter designated the electromagnetic signal characteristic of the biological activity of the analytical sample, and

- the detection and/or the measurement of the ligand-receptor complexes formed during the reaction between the two reagents.

63. (New) A detection process according to claim 62, characterised in that the concentrations of the ligand and of the receptor are chosen so as to be sufficient to lead to the obtaining of ligand-receptor complexes detectable in the absence of the application of said electromagnetic signal characteristic of the biological activity of the analytical sample, but lower than the concentrations likely to lead to a saturation of the reaction between this ligand and this receptor.

64. (New) A detection process according to claim 61, characterised in that it includes:

- the bringing into contact of the analytical sample with a reagent containing either the receptor, if the substance sought in the sample is the ligand, or the ligand, if the substance sought in the sample is the receptor, in conditions suitable to allow their reaction,
- previously, simultaneously or subsequently to this bringing into contact, the application, to this sample and/or this reagent, of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair, and
- the detection and/or the measurement of the ligand-receptor complexes possibly formed.

65. (New) A device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it implements a process according to claim 61, and in that it comprises:

- a) reception means (47) of the analytical sample and of a reagent containing either the receptor, or the ligand, allowing them to be brought into contact in conditions suitable to allow their reaction;

- b) a source (5, 9, 9', 19) of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair;

- c) application means (51) to the sample and/or to the reagent of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of the ligand-receptor pair delivered by said source (5, 9, 9', 19); and

- d) detection and/or measurement means (53, 55, 57) of the ligand-receptor complexes formed during the reaction between the sample and the reagent.

66. (New) A device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it implements a process according to claim 61, and in that it comprises:

- a) reception means (47) of the analytical sample and of a reagent containing respectively the ligand and the receptor, allowing them to be brought into contact in conditions suitable to allow their reaction;

- b) acquisition means of an electromagnetic signal obtained from an electrical signal produced by a sensor placed in front of the analytical sample; said electromagnetic signal being hereinafter designated the electromagnetic signal characteristic of the biological activity of the analytical sample, and

- c) application means (51) to one and/or the other of the reagents of said electromagnetic signal characteristic of the biological activity of the analytical sample, and

- d) detection and/or measurement means (53, 55, 57) of the ligand-receptor complexes formed during the reaction between the two reagents.

67. (New) A device according to claim 65, characterised in that the detection means comprise optical detection means.

68. (New) A device according to claim 65, characterised in that it comprises an enclosure (13) fitted with an electrical and magnetic shielding surrounding said reception means (47).

69. (New) Application of a process for detecting the presence of a substance in an analytical sample according to claim 60 to biological diagnostics in human or veterinary medicine.

70. (New) Application of a process for detecting the presence of a substance in an analytical sample according to claim 61 to bacteriological control in the pharmaceutical industry, the cosmetics industry, food production and industries.

71. (New) A process for detecting the presence, in an electromagnetic test signal, of the electromagnetic signal characteristic of the biological activity of one and/or the other of the two elements of a ligand-receptor pair; characterised in that it includes the implementation of an amplification process according to claim 42.

72. (New) A detection process according to claim 71, characterised in that the electromagnetic signal is the electromagnetic signal radiated by an electromagnetic radiation source.

REMARKS

The above preliminary amendment is made to add new claims and remove multiple dependencies from claims 6, 7, 8, 11, 13, 14, 15, 17, 18, 20, 26, 27, 28, 29 and 30.

Applicants respectfully request that the preliminary amendment described herein be entered into the record.

If a telephone conference would be helpful in resolving any issues concerning this communication, please contact Applicants' primary attorney-of record, John J. Gresens (Reg. No. 33,112), at (612) 371.5265.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: February 6, 2001

By _____
John J. Gresens
Reg. No. 33,112

JJG/tvm

AN AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USES

The present invention relates to an amplification process of the formation of complexes between the two elements of a ligand-receptor pair, to a process and to a device for detecting the presence, in a sample (hereinafter "analytical sample"), of a substance corresponding to one of the two elements of a ligand-receptor pair, implementing this amplification process, to the applications of this detection process, and to a process for detecting the presence, in an electromagnetic signal, of the electromagnetic signal characteristic of the biological activity of a substance corresponding to one of the two elements of a ligand-receptor pair, also implementing said amplification process.

To detect the presence of a substance in an analytical sample, very many methods have been suggested based on the capacity of this substance to bind itself specifically to one substance and to react with it.

In particular, the affinity properties presented by antibodies in respect of antigens are at the basis of a great number of immunological detection methods which in common use the formation of antigen-antibody complexes - the substance sought being able to be either the antigen, or the antibody - and detect, indeed quantify, the complexes so formed.

As examples of immunological detection methods which are very frequently used, may be mentioned immunoprecipitation, agglutination reactions, equilibrium dialysis, fluorescence suppression, fluorescence polarisation, immunoelectrophoresis, counter immunoelectrophoresis or electrosyneresis,

radioimmunoassay (RIA), enzyme immunoassay (ELISA) or else immunofluorescence.

These immunological detection methods, if indisputably they have good qualities, are not however entirely satisfactory.

In the first place, their sensitivity (which is defined by the minimum concentration of substance sought which these methods detect) is, in most cases, insufficient. Thus, BERZOFSKY and BERKOWER (*Antigen-Antibody Interaction*, In: WE Paul, *Fundamental Immunology*, RAVEN press, New York, 1984, 595) have shown that, as far as the detection of antibodies for example is concerned, with the exception of bacteriophage neutralisation tests with which it is possible to detect the presence of a single molecule of antibody but the use of which is extremely limited, very few methods have a sensitivity lower than 10 ng of antibody per ml of sample.

It is therefore desirable to develop new techniques which allow the detection threshold of a sought substance to be lowered.

Furthermore, all the immunological detection methods hitherto proposed include a stage which consists in incubating a pre-set volume - which is generally at the minimum of 500µl - of the sample for analysis with a specific reagent and to do this, for each substance sought. For this reason, they have the drawback of requiring, as soon as the analysis of a sample involves several substances - as is often the case in medical analyses for diagnostic purposes - a sample of relatively large volume, which is not always easily tolerated by patients, particularly in the case of blood samples.

Moreover, the fact that these detection methods require, for their implementation, the availability of the sample for analysis or, at the very least, of a specimen of it, is not without imposing a certain number of constraints. Indeed:

- on the one hand, samples which have been subjected to analysis frequently have to be preserved so that the reliability of these analyses may subsequently be monitored or for additional analyses to be made. So, for example, blood transfusion centres, forensic medicine services and tissue sampling centres preserve specimens of all the biological samples that they are called on to take. This preservation, which is made by freezing said specimens, apart from being not insignificant in cost, requires adapted equipment and premises.

- on the other hand, samples can rarely be analysed at the place where they have been taken and it is often necessary to take them to the laboratory responsible for analysing them. In fact, transporting biological samples, apart from this never being very easy to implement given the short preservation period of biological substances in the absence of freezing, poses a certain number of difficulties when these samples are potentially contaminant. Moreover, the length of time taken to transport them differs by as much the obtaining of results from the analysis.

The problem arises, as a consequence, of supplying a method which makes it possible to detect the presence of a substance in a sample with, at the same time, very high sensitivity and high specificity, while offering the possibility of carrying out as many analyses as necessary from micro-samples, and to be free, moreover, from the constraints of preservation, despatch, and transportation

of the samples presented by currently used methods for the detection of a substance, and which can, additionally, be implemented easily and rapidly without requiring heavy and expensive equipment.

In fact, in the context of their work on the transmission of a biological activity in the form of an electromagnetic signal, the Inventors have noted that the effect of applying, to one and/or the other of the elements of a ligand-receptor pair such as an antigen-antibody pair, the electromagnetic signal characteristic of the biological activity of one and/or the other of these elements is, quite surprisingly, to amplify the formation of complexes between the two elements of this pair when these latter are set to react together, and this, very specifically, and have had the idea of capitalising on this effect in order to detect on the one hand, the presence of a substance in an analytical sample and, on the other hand, the presence of the electromagnetic signal characteristic of the biological activity of a substance in an electromagnetic radiation.

An object, therefore, of the present invention, is a process amplifying the formation between the two elements of a ligand-receptor pair by reaction of these two elements, which process is characterised in that it includes:

- bringing into contact the two elements of the ligand-receptor pair in conditions suitable to allow their reaction, and

- previously, simultaneously or subsequently to this bringing into contact, the application to one and/or the other of these elements of the electromagnetic signal characteristic of the biological activity of one and/or the other of said elements.

In terms of the present invention, by "ligand-receptor pair" is understood any pair formed by two substances capable of recognising each other specifically, of binding and of reacting together forming complexes. Thus, it may be an antigen-antibody pair or a hapten-antibody pair in which the ligand (the antigen or the hapten) can be a biological compound (protein, enzyme, hormone, toxin, tumour marker, etc.), a chemical compound (medicinal active ingredient for example), or a cellular or particle antigen (cell, bacterium, virus, fungus, etc.), the receptor being able to be a soluble antibody or a membranous receptor. It may also be a pair formed by an enzyme or its specific substrate.

Furthermore, by "electromagnetic signal characteristic of the biological activity" of an element is understood the electromagnetic signal picked up from a biologically active element such as a substance, a cell or a micro-organism, etc., or from a material containing this element such as a purified preparation, a biological sample, an organ or a living being, as has been described in International Application WO 94/17406 in the name of J. BENVENISTE. By "electromagnetic signal characteristic of the biological activity" of an element is also understood the signals derived from a signal as defined above by signal digitisation and/or processing. Furthermore, in this expression, the term "characteristic" is used in the sense that the picked up electromagnetic signal contains information characterising the fact that the material from which this signal is picked up shows the biological activity in question. The electromagnetic signal picked up from a material containing a plurality of biologically active

elements shows the biological activity of each of the elements that it contains.

According to a preferred first mode of implementation of the amplification process according to the invention, the reaction between the ligand and the receptor is obtained by using two reagents containing respectively the ligand and the receptor, and to one and/or the other of these reagents is applied an electromagnetic test signal suspected to include the electromagnetic signal characteristic of the biological activity of this ligand and/or this receptor.

In what precedes and what follows, by the term "reagent" is denoted any preparation of which the composition is known, which contains the ligand or the receptor in an also known quantity and presents itself either in a dry form such as a lyophilisate to be reconstituted in a solvent, or in a liquid form such a solution or a suspension, the ligand and the receptor being able to be fixed on a solid phase (particles or beads of latex, glass or polystyrene etc.).

According to a first advantageous arrangement of this first mode of implementation, the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by exposure of a solution or a suspension containing one and/or the other of these reagents, to this electromagnetic signal.

Alternatively, the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by dilution of a solution or of a suspension including one and/or the other of these reagents, in a solvent having been previously exposed to this electromagnetic signal.

Thus, for example, when the reagents which it is required to use are in solution or in suspension in a liquid phase, it is possible to apply to them the electromagnetic test signal:

* either prior to their use:

- by exposing one and/or the other of these reagents or of the aliquots of one and/or the other of these reagents to this electromagnetic signal, or
- by diluting one and/or the other of said reagents or their aliquots in a volume of a solvent having been previously exposed to said electromagnetic signal,

* or during implementation of the amplification process according to the invention:

- by exposing to this electromagnetic signal an aliquot of each of these reagents, after placing these aliquots on a medium (plate for example) but prior to their being brought into contact, or
- by mixing an aliquot of the first reagent with an aliquot of the second reagent on a medium or in a tube, and by exposing this mixture to the electromagnetic signal, or else
- by mixing an aliquot of the first reagent with an aliquot of the second reagent on a medium or in a tube and by diluting this mixture in a volume of a solvent having been previously exposed to said electromagnetic signal.

According to another advantageous arrangement of this first mode of implementation, the application, to one and/or the other of the reagents, of the electromagnetic test signal is made by dissolution or putting into suspension this reagent or these reagents in a solvent having been previously exposed to this electromagnetic signal. This arrangement has a very

particular advantage when the reagents which it is desired to use are in a dehydrated form such as a lyophilisate, since it is then possible to apply the electromagnetic test signal to them simply by dissolving them or by putting them in suspension depending on the case, in a volume of a solvent having been previously exposed to said electromagnetic signal.

To advantage, the electromagnetic test signal is an electromagnetic signal picked up from an analysis sample suspected to contain this ligand and/or this receptor, this sample being able to stem just as well from a biological sample (blood, urine, milk, etc.) as from a non biological sample (water, food product, pharmaceutical product, cosmetic product, etc.).

Alternatively, the electromagnetic test signal can also be an electromagnetic signal radiated by an electromagnetic radiation source, particularly a source suspected to emit radiation harmful to living beings such as the high voltage transmission line, transformer, electric motor, micro-wave oven, particle accelerator, X-ray source etc. Likewise, the electromagnetic test signal can stem from the acquisition of a mechanical signal like vibrations, an electrostatic signal or the like.

According to a second preferred mode of implementation of the amplification process according to the invention, the reaction between the ligand and the receptor is obtained by bringing into contact an analysis sample suspected to contain the ligand and/or the receptor, with a reagent containing either the receptor, or the ligand (according to the substance suspected to be present in the analytical sample with which it is desired to make this reagent react), and, to this sample and/or to this reagent, is applied the electromagnetic signal

characteristic of the biological activity of said ligand and/or said receptor.

According to a first advantageous arrangement of this second mode of implementation, the application, to the analysis sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor is made by exposure of this sample to this electromagnetic signal or signals, or by dilution of this sample in a solvent having been previously exposed to said electromagnetic signal or signals.

According to another advantageous arrangement of this second mode of implementation, the application, to the reagent intended to react with the analysis sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor is made by exposure of a solution or a suspension containing this reagent to this electromagnetic signal or signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to this electromagnetic signal or signals, or again by dissolution or putting into suspension of this reagent in a solvent having been previously exposed to said electromagnetic signal or signals.

Alternatively, to the analysis sample and to the reagent intended to react with it, is applied the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, by exposure of a solution or a suspension containing this sample and this reagent to this electromagnetic signal or signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to said electromagnetic signal or signals.

According to a particularly preferred arrangement of this second mode of implementation, to the analysis sample and/or to the reagent intended to react with it, is applied both the electromagnetic signal characteristic of the biological activity of the ligand and the electromagnetic signal characteristic of the biological activity of the receptor. Indeed, the Inventors have noted that, if it is enough to apply, to the elements of the ligand-receptor pair, the electromagnetic signal characteristic of the biological activity of a single one of these elements to obtain an amplification of the complexes formed by their reaction, this amplification is higher when the electromagnetic signals characteristic of the biological activity of each of them are applied to these elements simultaneously.

Whatever the mode of implementation of the amplification process according to the invention, the solvent having been previously exposed to the electromagnetic signal or signals is to advantage water or physiological solute.

Reagents able to be used in the amplification process according to the invention and containing the ligand on the one hand, and the receptor on the other hand, can just as well be ready-to-use commercially available reagents as reagents specially designed and prepared for the implementation of this process. Apart from the fact that, as mentioned above, these reagents can come in different forms (dry, liquid, etc.), they can, furthermore, be coupled to a marker such as a radioactive isotope, an enzyme, a fluorescent substance, a coloured particle, biotin or an organometallic compound, suitable to allow detection and/or measurement

of the ligand-receptor complexes resulting form the reaction between the ligand and the receptor.

The amplification process includes to advantage, moreover, an acquisition stage of the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair.

As previously indicated, the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair can stem either from an analysis sample suspected to contain this element or elements, or from an electromagnetic radiation source or from the acquisition of a mechanical (vibrations), electrostatic or other signal, or again from reagents containing the ligand or the receptor in solution or in suspension in a solvent, according to the modes of implementation of the amplification process according to the invention.

In a particularly advantageous way, the amplification process according to the invention also includes a recording and restitution stage of information representative of the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair. Thus, the electromagnetic signal characteristic of the biological activity of an analytical sample, once recorded, can be preserved indefinitely and used as often as necessary. Similarly, the electromagnetic signals characteristic of the biological activity of the ligand and of the biological activity of the receptor picked up from reagents, can be recorded once and for all and be used to obtain a plurality of reactions involving this ligand and this receptor.

The amplification process additionally includes to advantage a stage of detection of the complexes resulting from the reaction between the ligand and the receptor and, possibly, of measurement of these complexes. This stage can, to advantage, be completed by comparing the results obtained with those observed for a reaction serving as a "reference", that is to say a reaction conducted with the same ligand-receptor pair and in the same reaction conditions, but without application of an electromagnetic signal to the elements of this pair, whether previously, simultaneously or subsequently to their being brought into contact.

The detection and/or measurement of the ligand-receptor complexes are able to be carried out by all the methods conventionally used to reveal and quantify the formation of such complexes. Thus, in the case of antigen-antibody complexes, it is possible just as well to use a revelation by agglutination, by immuno-precipitation, by fluorescence suppression, by fluorescence polarisation as a radio-immunological, immunoenzymatic test or else an immuno-fluorescence test.

According to a particularly preferred mode of implementation of the amplification process according to the invention, the ligand is an antigen or a hapten, whereas the receptor is an antibody or a membranous receptor targeted specifically against this ligand.

In a particularly advantageous way, the reaction between this ligand and this receptor is a reaction revealed by agglutination, given its simplicity and its speed of execution.

Another object of the present invention is a process for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in

an analytical sample, characterised in that it includes the implementation of an amplification process as defined above.

According to a particularly preferred first mode of implementation of this detection process, this includes:

- the bringing into contact of two reagents containing respectively the ligand and the receptor, in conditions suitable to allow their reaction,

- previously, simultaneously or subsequently to this bringing into contact, the application, to one and/or the other of these reagents, of the electromagnetic signal characteristic of the biological activity of the analytical sample, and

- the detection and/or the measurement of the ligand-receptor complexes formed during the reaction between the two reagents.

Thus, obtaining an amplification of the formation of ligand-receptor complexes between the two reagents relative to a "reference" reaction (as previously defined) conveys the presence, in the electromagnetic signal of the biological activity of the analysis sample, of the electromagnetic signal characteristic of the biological activity of the substance sought and, as a consequence, conveys the presence, in this sample, of the substance sought.

In the event of such amplification being obtained and the analytical sample being able to contain not just one of the two elements of the ligand-receptor pair, but these two elements, the presence, in this sample, of the substance sought can be confirmed by comparing the results obtained with:

- either those observed for a reaction conducted in the same reaction conditions but with an application both

of the electromagnetic signal characteristic of the biological activity of the analysis sample and of the electromagnetic signal characteristic of the biological activity of the ligand,

- or those observed for a reaction conducted in the same reaction conditions but with an application both of the electromagnetic signal characteristic of the biological activity of the analysis sample and of the electromagnetic signal characteristic of the biological activity of the receptor.

Thus, if the simultaneous application of the electromagnetic signal characteristic of the biological activity of the analytical sample and of the electromagnetic signal characteristic of the biological activity of the ligand is conveyed by an amplification of the formation of ligand-receptor complexes compared with the application of the single electromagnetic signal characteristic of the biological activity of said analytical sample, then this means that this sample does not contain a ligand and therefore contains only the receptor. It being the absence of an increase in the formation of ligand-receptor complexes that signals the presence of the ligand in the analytical sample.

Similarly, if the simultaneous application of the electromagnetic signal characteristic of the biological activity of the analytical sample and of the electromagnetic signal characteristic of the biological activity of the receptor is conveyed by an amplification of the formation of ligand-receptor complexes compared with the application of the single electromagnetic signal characteristic of the biological activity of said analytical sample, then it may be inferred from this that this sample does not contain a receptor and therefore

contains only the ligand. It being the absence of an increase in the formation of ligand-receptor complexes that signals the presence of the receptor in the sample.

To avoid getting falsely negative results, that is to say results which would not make it possible to reveal an amplification effect of the application of the electromagnetic signal characteristic of the activity of the analytical sample and this, even though the latter contains in reality the substance sought, the concentrations of the ligand and of the receptor set to react are chosen to advantage so as to be sufficient to lead to the obtaining of ligand-receptor complexes detectable in the absence of the application of the electromagnetic signal characteristic of the biological activity of said sample, but lower than the concentrations able to lead to a saturation of the reaction between this ligand and this receptor.

According to a second preferred mode of implementation of this detection process, this includes:

- the bringing into contact of the analytical sample with a reagent containing either the receptor, if the substance sought in the sample is the ligand, or the ligand, if the substance sought in the sample is the receptor, in conditions suitable to allow their reaction,
- previously, simultaneously or subsequently to this bringing into contact, the application, to this sample and/or this reagent, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, and
- the detection and/or the measurement of the ligand-receptor complexes possibly formed, in which case, the obtaining of ligand-receptor complexes conveys the

presence of the substance sought in the analytical sample.

This second preferred mode of implementation has a very particular advantage in detecting the presence of substances in samples, about which it is known that they are not detectable or only with great difficulty by the other available detection methods, since these substances are generally present in very low concentrations, indeed at trace level.

The process for detecting the presence of a substance in an analytical sample according to the invention has numerous advantages.

Indeed, on the one hand, it makes it possible to detect the presence of a sought substance with very great sensitivity and high specificity. Therefore, in the case, for example, of a bacteriological analysis, it makes it possible to eliminate the need to isolate the different germs, to cultivate them, to make an antibiogramme and to identify these germs by their biochemical, morphological and immunological character, and means that results can be obtained more rapidly than by the immunological detection methods currently used in bacteriology.

On the other hand, to the extent that it is enough to have a sample of the size of a drop to be in a position to acquire and record the electromagnetic signal characteristic of the biological activity of this sample and that, this signal, once recorded can be restored on request, this process offers the possibility of making as many analyses as desired from a microsample.

Lastly, the recording of an electromagnetic signal being able to be preserved indefinitely, for example in the form of an information file able to be preserved on a simple diskette or a CD-Rom, and to be transmitted from

one place to another by any digital data transmission means, this process makes it possible, moreover, to eliminate all the constraints of preservation, despatch and transportation of samples presented by currently used methods for the detection of a substance.

This process is able to be used to detect any substance capable of binding specifically with another substance and of reacting with it, it being understood that the term "substance" as it is used here, denotes just as well a biological compound, a chemical compound, a cell as a micro-organism of the bacterium, virus or fungus type, knowing particularly that for any hapten, protein or protein complex, it is possible to find on the market or to have manufactured the corresponding antibodies. By this token, this process finds, particularly, application in biological diagnostics, whether in human or veterinary medicine, or for the control of bacteriological quality in industries such as the pharmaceutical industry, the cosmetics industry, food production and industries.

A further object of the present invention is a process for detecting the presence, in an electromagnetic test signal, of an electromagnetic signal characteristic of the biological activity of a substance corresponding to one of the two elements of a ligand-receptor pair, which process is characterised in that it includes the implementation of an amplification process as defined above.

According to a preferred mode of implementation of this detection process, the electromagnetic test signal is the electromagnetic signal radiated by an electromagnetic radiation source.

Another object of the invention is a device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, which device is characterised in that it implements a process according to the invention and in that it comprises:

- a) reception means of the analytical sample and of a reagent containing either the receptor, or the ligand, allowing them to be brought into contact in conditions suitable to allow their reaction;

- b) an electromagnetic signal source characteristic of the activity of the ligand and/or of the receptor;

- c) application means of the signal delivered by said electromagnetic signal source to the sample and/or the reagent; and

- d) detection and/or measurement means of the ligand-receptor complexes formed during the reaction between the sample and the reagent.

A further object of the invention is a device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, which device is characterised in that it implements a process according to the invention and in that it comprises:

- a) reception means of two reagents containing respectively the ligand and the receptor, allowing them to be brought into contact in conditions suitable to allow their reaction;

- b) acquisition means of an electromagnetic signal of the analytical sample;

- c) application means of the signal delivered by said electromagnetic signal acquisition means to one and/or the other of the reagents; and

- d) detection and/or measurement means of the ligand-receptor complexes formed during the reaction between the two reagents.

According to an advantageous embodiment of these devices, the detection means comprise optical detection means.

In a preferred way, these devices comprise an enclosure fitted with an electrical and magnetic shielding surrounding said reception means.

Apart from the preceding arrangements, the invention includes still other arrangements which will emerge from the following supplementary description, which relates to embodiment examples of signal acquisition, recording and application devices able to be used according to the invention and to examples of experiments having allowed the amplification process object of the present invention to be validated, and which refers to the appended drawings in which:

- Figure 1 shows a diagram of a first embodiment example of a signal acquisition device able to be used according to the present invention;

- Figure 2 shows a diagram of a second embodiment example of a signal acquisition device able to be used according to the present invention;

- Figure 3 shows a diagram of a first embodiment example of a signal recording device able to be used according to the present invention;

- Figure 4 shows a diagram of a second embodiment example of a signal recording device able to be used according to the present invention;

- Figure 5 shows a diagram of an embodiment example of a signal application device able to be used according to the present invention;

- Figure 6 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after application of the electromagnetic signal characteristic of the biological activity of *Streptococcus*;

- Figure 7 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli*;

- Figure 8 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after simultaneous application of the electromagnetic signals characteristic of the biological activity of *Streptococcus* and of the biological activity of an antibody targeted against *Escherichia coli*;

- Figure 9 shows a black and white image of 320 pixels x 240 pixels of the agglutinates formed during an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen, after simultaneous application of the electromagnetic signals characteristic of the biological activity of *Escherichia coli* and of the biological activity of its specific antibody; and

- Figure 10 shows a diagram of an embodiment example of a detection and/or measurement device of the ligand-

receptor complexes able to be used according to the present invention.

In Figures 1 to 5 and 10, the same references have been used to denote the same elements.

Furthermore, each image in figures 6 to 9 corresponds to a surface of about 2 mm x 1·5 mm of the medium on which the agglutination reactions have been obtained.

It must be clearly understood, however, that these examples are given only as illustrations of the object of the invention and in no way constitute a restriction of it.

Reference is made first of all to Figures 1 to 5.

In Figure 1, a first embodiment example has been shown diagrammatically of an acquisition device of the electromagnetic signal characteristic of the biological activity of a substance 1 placed in a container 3, for example a test tube. A sensor 5, typically a coil of the "telephone sensor" type marketed for the purpose of being applied to a telephone receiver and connected to a tape recorder, is applied against the container 3. The container 3 can also be constituted by a biological wall, particularly the skin of a living being. In such a case, the acquisition of the electromagnetic signal is made in a non-invasive way.

The signal picked up by the coil 5 is, to advantage, amplified by an amplifier 7 and is available at an output terminal 9. Without this presenting any kind of restrictive character of the example shown, a first end of the coil 5 is connected to the input of the amplifier-preamplifier 7, the opposite end being connected to a mass 11. In an embodiment example, the coil 5 is a commercially available telephone sensor having a length

of 6 mm, an internal diameter of 6 mm containing a metal core, an external diameter of 16 mm and an impedance of 300 Ω.

In Figure 2 has been shown diagrammatically the preferred embodiment example of an acquisition device of the electromagnetic signal characteristic of the biological activity of a substance 1 contained in a container 3, in which the device includes, preferably, in an enclosure 13 fitted with an electrical and magnetic shielding, an irradiation transducer 15 of said substance 1 powered by a generator 17. The transducer 15 comprises, for example, a coil, to advantage completed by wave guides, for example an air gap (not shown) placed in contact with the external walls of the container 3.

The generator 17 generates a low frequency sinusoidal signal, low frequency square waves, pink noise or, to advantage, white noise. The excitation signal spectrum feeding the coil 15 corresponds approximately to the spectrum of audible frequencies (20 Hz - 20 000 Hz). The generator 17 can be an analogue signal generator of known type or, for example, a read-only memory (ROM, PROM, EPROM, EEPROM in the terminology of the English-speaking world) containing the digital signal of the desired noise and which is connected to a digital-to-analogue converter, or the line output of a sound card of a multimedia micro-computer. However, the implementation of higher frequencies is not outside the framework of the present invention.

The acquisition sensor 5 can comprise a coil similar to the coil 5 of the device in Figure 1 or, to advantage, a small diameter coil connected by an electromagnetic wave guide to the wall of the container 3. To advantage,

the signal picked up by the sensor 5 is available to an output terminal 9 of an amplifier-preamplifier 7.

The signal available at the terminal 9 may be directly applied to the substance or substances to be irradiated, particularly to the ligand, to the receptor or to the ligand-receptor pair (particularly by means of the device shown in Figure 5 and described below).

Recording the signal can be carried out in analogue by a signal recorder 19 (Figure 3), particularly on magnetic tape 21 adapted to the frequencies of the signal picked up. For acoustic frequencies, a tape recorder may particularly be used. The output terminal 9 of the signal acquisition device in Figures 1 or 2 is connected to the microphone input or to the line output of such a tape recorder. During playback, the signal is picked up at an output terminal 9', particularly at the line output or at the tape recorder loudspeaker output 19.

To advantage, a digital recording is made after analogue-to-digital conversion of the signal. A micro-computer 23 shown in Figure 4, fitted with a signal acquisition card 25, is used for example. This can be for example a computer of the PC type, operating under the WINDOWS® 95 operating system of the MICROSOFT Company and comprising, apart from the acquisition card 25, a micro-processor 27, an input/output interface 29, a controller 31 of a file store 33 and a video interface 35 connected by one or more buses 37. The acquisition card 25 comprises an analogue converter 39 having, preferably, a resolution above 10 bits, for example equal to 12 bits, as well as a sampling frequency double the maximum frequency that it is wished to be able to digitise for the processing of signals. In the acoustic frequencies, the sampling frequency is to advantage approximately

equal to 44 kHz. To process these types of signal, a micro-computer sound card is used to advantage, for example the Soundblaster 16 or the Soundblaster 32 card sold by the CREATIVE LABS Company. The computer 23 fitted with the restitution acquisition card 25, particularly of a Soundblaster 32 card can to advantage replace the signal generator 17 in Figure 2.

The output 9 of the signal acquisition devices in Figures 1 is connected to the input 9 of the analogue-to-digital converter 39 of the card 25 of the computer 23; the signal is then acquired for a period for example of between 1 and 60 s and the digital file is recorded in a file store 33 for example in the form of a WAV format sound file. This file may possibly be subject to digital processing, like for example a digital amplification for calibration of the signal level, a filtering for the elimination of undesired frequencies, or be converted into its spectrum by a discrete FOURIER transform, preferably by the fast FOURIER transform algorithm (FTT in the terminology of the English speaking world).

The sound reproduction time can be increased by repeating in a file several times a fragment or the totality of the original sound file.

On command, the possibly processed file is converted by a digital-to-analogue converter 41 of the card 25 (or of a separate card), which delivers at the output 9' the electromagnetic analogue signal characteristic of the biological activity to be applied, according to the amplification process according to the invention, for example to an aliquot 43 of a first reagent and to an aliquot 45 of a second reagent, as shown in Figure 5.

To advantage, the application of the signal to these aliquots is made prior to their mixture. The medium on

which these aliquots are placed, for example, a plate 47 fitted with a capillary 49 in the form of a coil, is placed in an electromagnetic field radiated by a transducer 51, typically a coil a first end of which 9,9′ is connected to the output 9 of an acquisition device of Figures 1 or 2 or to the output 9′ of a recording device of Figures 3 or 4. The coil end opposite the connection terminal 9,9′ is, for example, connected to the mass 11.

Without this having any kind of restrictice character, the transducer 51 comprises to advantage a coil, of horizontal axis, allowing the introduction of a plate 47. The coil has, for example, a length of 120 mm, an internal diameter of 25 mm, an external diameter of 28 mm, has 631 revolutions of a wire of a diameter of 0·5 mm and a resistance of 4·7 Ω.

To advantage, the electrical signal applied to this coil 51 will have an amplitude of 2 effective volts.

EXAMPLE 1: AMPLIFICATION OF THE FORMATION OF AGGLUTINATES BETWEEN THE POLYSACCHARIDIC ANTIGEN OF *ESCHERICHIA COLI* K1 AND AN ANTIBODY TARGETED AGAINST THIS ANTIGEN

The amplification process according to the invention has been validated by testing the effects, on an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and an antibody targeted against this antigen:

- of the application of the electromagnetic signal characteristic of the biological activity of an antigenic substance alien to this reaction such as *Streptococcus*,

- of the application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli*,

- of the simultaneous application of the electromagnetic signal characteristic of the biological activity of *Streptococcus* and of the electromagnetic signal characteristic of the biological activity of an antibody targeted against *Escherichia coli,* and lastly

- of the simultaneous application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* and of the electromagnetic signal characteristic of the biological activity of an antibody targeted against this antigen.

1) **Conducting tests:**

a) Acquisition of electromagnetic signals:

The acquisition of the electromagnetic signal characteristic of the biological activities of *Streptococcus, Escherichia coli* and of its specific antibody was made by means of the recording material in Figure 2.

The acquisition of the electromagnetic signal characteristic of the biological activity of *Streptococcus* was effected by placing at the centre of the enclosure 13 a tube containing 1 ml of an aqueous suspension of previously formolated *Streptococcus* bacteria (6.10^6 bacteria/ml).

The acquisition of the electromagnetic signals characteristic of the biological activity of *Escherichia coli* and of its specific antibody was made by operating in the same way, but by using respectively:

- a tube containing 1 ml of an aqueous suspension of previously formolated *Streptococcus* bacteria (6.10^6 bacteria/ml).

- a tube containing 1 ml of a suspension of particles of a latex sensitised by a specific monoclonal mouse antibody of *Escherichia coli* K1, coming from a

PASTOREX® MENINGITIS kit (Reference 61709 - SANOFI DIAGNOSTICS PASTEUR).

b) Preparation of the reagents of the agglutination reaction:

The tests were conducted by using as reagents:

- on the one hand, a solution of polysaccharidic antigen of *Escherichia coli* K1 prepared by dissolution of an antigen extract coming from a PASTOREX® MENINGITIS kit (Reference 61709 - SANOFI DIAGNOSTICS PASTEUR) in 1 ml of distilled and sterile water, then dilution to 1/7, 1/7·5, or 1/8 in a physiological serum; and

- on the other hand, the latex sensitised by a specific monoclonal mouse antibody of the antibody of *Escherichia coli* K1 present in this same kit, after dilution to 1/3 in a physiological serum.

c) Application of the electromagnetic signals to the agglutination reaction

For each of the tests, the following protocol was used:

- into an oven heated to 37°C is placed a transducer constituted by a coil measuring 120 mm in length and 25 mm in internal diameter, having 631 revolutions and a resistance of 4·7 Ω, and connected to the output 9′ of the digital-to-analogue converter 41 of a Soundblaster card of a computer 23 restoring the recording files constituted by the electromagnetic signal that it is desired to apply, the time needed to bring this transducer to the temperature of 37°C;

- on a plate fitted with a capillary in the form of a coil (of the type provided in PASTOREX MENINGITIS kits), at a short distance from the opening of the latter, is put a drop (i.e. 40 to 50 µl) of the antigen solution as described at point b) above, and a drop

(corresponding also to a volume of 40 to 50 µl) of the latex sensitised by the antibody, taking care that these drops do not mix together;

- to the two drops of reagents so placed, is applied the electromagnetic signal or signals desired by placing the plate at the centre of the transducer for about 2 mn and by restoring a sound file by means of the computer 23 in figure 4,

- the two drops of reagents are mixed for about 10 seconds and for about 13 minutes in the oven the reaction mixture is left to migrate in the capillary and the agglutination reaction is left to happen;

- the plate is then taken out of the oven and this agglutination is then read.

As can be seen in Figure 10, this reading is taken by analysis, using analysis and image processing software run on a computer of the PC type 23' operating on the WINDOWS® 95 (MICROSOFT) operating system, of an image acquired using a video camera 53 positioned on an optical microscope 55 and connected to said computer by a video acquisition card 57. The camera 53 works in levels of grey. A first processing increasing the contrast, the threshold being adjusted so that the agglutinates appear as black, whereas the zones devoid of particles of latex or of agglutinates appear as white.

From the analysis of the bidimensional spatial distribution of the dark zones of the image, the computer determines an agglutination index (I) calculated according to the formula:

$$I = \frac{\text{Surface taken up by agglutinates larger than 60 pixels}}{\text{Surface taken up by agglutinates equal to or smaller than 60 pixels}}$$

This agglutination index is higher in proportion to the size of the agglutinates formed during the agglutination reaction. The amplification is considered as positive when, during an experiment, the application of the electromagnetic signals characteristic of the biological activity of *Escherichia coli* and/or of the biological activity of its specific antibody leads to an agglutination index being obtained at least greater by 40% than the maximum agglutination index obtained, in the same conditions, and out of for example 3 experiments, after application of the electromagnetic signal characteristic of the biological activity of *Streptococcus*.

2) **Results:**

Table 1 below shows the agglutination indexes (I) obtained in a first series of tests aiming to compare the effects of the application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* to those observed after application, in the same reaction conditions, of the electromagnetic signal characteristic of the biological activity of *Streptococcus*, and this, for 3 different dilutions (1/7, 1/7·5, or 1/8) of the solution of polysaccharidic antigen of *Escherichia coli* K1 used as a reagent in the agglutination reactions.

TABLE 1

Dilution of the E. coli K1 antigen solution	Agglutination index (I)	
	Streptococcus Signal	*E. coli* Signal

1/7	11 6 16	173 52 154
1/7·5	58 32 12	141 117 107
1/8	10 6 8	113 37 21

Furthermore, Figures 6 and 7 show, by way of examples, images of the agglutinates formed on the one hand, after application of the electromagnetic signal characteristic of the biological activity of *Streptococcus* (Figure 6) and, on the other hand, after application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* (Figure 7). These images correspond respectively to the agglutination indexes of 32 and 117 which are reported on the 5th line of results on Table 1.

Table 2 below shows, in its turn, the agglutination indexes (I) obtained in a second series of experiments in the context of which the effects of the simultaneous application of the electromagnetic signal characteristic of the biological activity of *Escherichia coli* and of the electromagnetic signal characteristic of the biological activity of the antibody targeted against *Escherichia coli,* were compared with those of the simultaneous application, in the same reaction conditions, of the electromagnetic signal characteristic of the biological activity of *Streptococcus* and of the electromagnetic signal characteristic of the biological activity of the antibody targeted against *Escherichia coli,* and this, for 2 different dilutions (1/7, and 1/7·5) of the solution of

polysaccharidic antigen of *Escherichia coli* K1 used as a reagent.

TABLE 2

Dilution of the E. coli K1 antigen solution	Agglutination index (I)	
	Streptococcus Signal + Anti-E.coli antibody Signal	*E. coli* Signal + Anti-E.coli antibody Signal
1/7	18 71	94 247
1/7·5	48 93	212 1141

Figures 8 and 9 show, also by way of example, images of the agglutinates which correspond respectively to the agglutination indexes of 71 and 247 reported on the 2nd line of results in Table 2.

All these results clearly prove the aptitude shown by the electromagnetic signal characteristic of the biological activity of one element of a ligand-receptor pair, to amplify the formation of complexes formed by the reaction between this ligand and this receptor and this, very specifically, since the electromagnetic signal characteristic of the biological activity of an element which is biologically active but alien to this reaction does not produce an amplification effect.

They also show that this amplification is especially pronounced when, to the two elements of the ligand-receptor pair, is applied both the electromagnetic signal characteristic of the biological activity of this ligand

and the electromagnetic signal characteristic of the biological activity of this receptor.

EXAMPLE 2: DETECTION OF THE PRESENCE OF ESCHERICHIA COLI IN A SAMPLE

The advantage of using the amplification process according to the invention to detect a substance present in an analytical sample was verified by conducting a series of tests with the aim of comparing the effects of the application, on an agglutination reaction between the polysaccharidic antigen of *Escherichia coli* K1 and a specific monoclonal mouse antibody of this antigen identical to that implemented in example 1 above, of the electromagnetic signal picked up from a sample of a food product, in the case in point stewed apples, previously contaminated by *Escherichia coli* bacteria, with those obtained during the application, in the same reaction conditions, of the electromagnetic signal picked up from a reference, or in other words uncontaminated sample of the same food product.

1) **Conducting tests:**

The acquisition of the electromagnetic signal of the samples of stewed apples (reference samples and contaminated samples) was made using the recording material in Figure 2, by placing at the centre of the enclosure 13:

- in the case of the reference samples, a tube containing 1 ml of stewed fruit previously diluted to 1/2 with physiological serum, and
- in the case of the contaminated samples, a tube containing 1 ml of stewed fruit previously diluted to 1/2 with physiological serum and contaminated, by addition of previously formolated *Escherichia coli* bacteria, at a rate of 3.10^6 bacteria per ml of diluted stewed fruit.

The tests were conducted by using as reagents:

- on the one hand, a suspension containing *Escherichia coli* bacteria previously formolated in physiological serum, at a rate of 10^7 bacteria/ml, and
- on the other hand, the latex sensitised by a specific mouse monoclonal antibody of the antibody of *Escherichia coli* K1 antibody present in this same kit, after dilution to 1/3 in physiological serum,

and by following an operating protocol identical to that described in paragraph c) of example 1 above.

2) **Results:**

Table 3 below shows the agglutination indexes (I) obtained in three series of tests.

TABLE 3

Tests	Agglutination index (I)	
	Reference samples	Contaminated samples
Series 1	10 25 27	42 93 104
Series 2	14 17 46	30 153 40
Series 3	19 34	54 314

As can be seen in Table 3, the size of the agglutinates formed during the reaction between the polysaccharidic antigen of *Escherichia coli* K1 and its specific antibody is substantially higher in the case where the electromagnetic signal applied during this reaction was picked up from a sample of stewed apples contaminated by *Escherichia coli* bacteria.

These results show that the amplification process according to the invention can to advantage be used to detect the presence, in an analytical sample, of a biologically active substance such as a bacterium, even when this sample has a complex composition, i.e. when it contains, as in the case of the samples of stewed apples, numerous other biologically active substances.

As emerges from what has been said previously, the Invention is in no way limited to the embodiments which have just been described in a more explicit way; it encompasses on the contrary all of its variants which can come to the mind of the technician in the field, without departing from the context, or the scope of the present Invention.

CLAIMS OF THE INTERNATIONAL PATENT

1. A process for amplifying a reaction between the two elements of a ligand-receptor pair, characterised in that it includes:

- the bringing into contact of the two elements of the ligand-receptor pair in conditions suitable to allow their reaction, and

- prior to, simultaneous with or subsequent to this bringing into contact, the application to one and/or the other of these elements of the electromagnetic signal characteristic of the biological activity of one and/or the other of said elements.

2. An amplification process according to claim 1, characterised in that the reaction between the ligand and the receptor is achieved by bringing into contact two regents containing respectively the ligand and the receptor, and to one and/or the other of these reagents is applied an electromagnetic test signal suspected to include the electromagnetic signal characteristic of the biological activity of this ligand and/or this receptor.

3. An amplification process according to claim 2, characterised in that the application, to one and/or the

other of the reagents, of the electromagnetic test signal is achieved by exposing a solution or a suspension containing one or the other of these reagents to this electromagnetic signal.

4. An amplification process according to claim 2, characterised in that the application, to one and/or the other of the reagents, of the electromagnetic test signal is achieved by diluting a solution or a suspension including one and/or the other of these reagents, in a solvent having been previously exposed to this electromagnetic signal.

5. An amplification process according to claim 2, characterised in that the application, to one and/or the other of the reagents, of the electromagnetic test signal is achieved by dissolving or putting into suspension this or these reagents in a solvent having been previously exposed to this electromagnetic signal.

6. An amplification process according to claim 4 or claim 5, characterised in that the solvent having been previously exposed to the electromagnetic signal characteristic of the biological activity of the analytical sample is water or physiological solute.

7. An amplification process according to any one of claims 2 to 6, characterised in that the electromagnetic test signal is the electromagnetic signal picked up from an analytical sample suspected to contain the ligand and/or the receptor.

8. An amplification process according to any one of claims 2 to 6, characterised in that the electromagnetic test signal is the electromagnetic signal radiated by an electromagnetic radiation source.

9. An amplification process according to claim 1, characterised in that the reaction between the ligand and

the receptor is achieved by bringing into contact an analytical sample suspected to contain the ligand and/or the receptor, with a reagent containing either the receptor, or the ligand, and to this sample and/or to this reagent is applied the electromagnetic signal characteristic of the biological activity of said ligand and/or of said receptor.

10. An amplification process according to claim 9, characterised in that the application, to the analytical sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor is achieved by exposing this sample to this or these electromagnetic signals, or by diluting this sample in a solvent having been previously exposed to said electromagnetic signal(s).

11. An amplification process according to claim 9 or claim 10, characterised in that the application, to the reagent intended to react with the analytical sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or of the receptor is achieved by exposing a solution or a suspension containing this reagent to this or these electromagnetic signals, or by diluting such a solution or suspension in a solvent having been previously exposed to this or these electromagnetic signals, or again by dissolving or putting into suspension this reagent in a solvent having been previously exposed to said electromagnetic signal(s).

12. An amplification process according to claim 9, characterised in that, to the analytical sample and to the reagent intended to react with it, is applied the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, by exposing a

solution or a suspension containing this sample and this reagent to this or these electromagnetic signals, or by diluting such a solution or suspension in a solvent having been previously exposed to said electromagnetic signal(s).

13. An amplification process according to any one of claims 9 to 12, characterised in that, to the analytical sample and/or to the reagent intended to react with it, is applied at one and the same time the electromagnetic signal characteristic of the biological activity of the ligand and the electromagnetic signal characteristic of the biological activity of the receptor.

14. An amplification process according to any one of claims 10 to 12, characterised in that the solvent having been previously exposed to the electromagnetic signal(s) is to advantage water or physiological solute.

15. An amplification process according to any one of claims 1 to 14, characterised in that it includes an acquisition stage of the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair.

16. An amplification process according to any one of claims 15, characterised in that it includes a stage for recording and retrieving data representing the electromagnetic signal characteristic of the biological activity of one and/or the other of the elements of the ligand-receptor pair.

17. An amplification process according to any one of claims 1 to 16, characterised in that it includes a stage for detecting and, possibly, for measuring the complexes resulting from the reaction between the ligand and the receptor.

18. An amplification process according to any one of claims 1 to 17, characterised in that the ligand is an antigen or a hapten, whereas the receptor is an antibody or a membranous receptor directed specifically against this ligand.

19. An amplification process according to claim 18, characterised in that the reaction between the antigen and the antibody or the hapten and the antibody is revealed by agglutination.

20. A process for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it includes the implementation of an amplification process according to any one of claims 1 to 7 and 9 to 19.

21. A detection process according to claim 20, characterised in that it includes:

- the bringing into contact of two reagents containing respectively the ligand and the receptor, in conditions suitable to allow their reaction,

- prior to, simultaneous with or subsequent to this bringing into contact, the application, to one and/or the other of these reagents, of the electromagnetic signal characteristic of the biological activity of the analytical sample, and

- the detection and/or the measurement of the ligand-receptor complexes formed during the reaction between the two reagents.

22. A detection process according to claim 21, characterised in that the concentrations of the ligand and the receptor are chosen so as to be sufficient to lead to the obtaining of ligand-receptor complexes detectable in the absence of the application of the

electromagnetic signal characteristic of the biological activity of said sample, but lower than the concentrations likely to lead to a saturation of the reaction between this ligand and this receptor.

23. A detection process according to claim 20, characterised in that it includes:

- the bringing into contact of the analytical sample with a reagent containing either the receptor, if the substance sought in the sample is the ligand, or the ligand, if the substance sought in the sample is the receptor, in conditions suitable to allow their reaction,

- prior to, simultaneous with or subsequent to this bringing into contact, the application, to this sample and/or this reagent, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, and

- the detection and/or the measurement of any ligand-receptor complexes that may have been formed.

24. A device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it implements a process according to claim 20 and in that it comprises:

a) reception means (47) of the analytical sample and of a reagent containing either the receptor, or the ligand, allowing them to be brought into contact in conditions suitable to allow their reaction;

b) an electromagnetic signal source (5, 9, 9', 19) characteristic of the activity of the ligand and/or the receptor;

c) application means (51) of the signal delivered by said electromagnetic signal source (5, 9, 9', 19) to the sample and/or the reagent; and

d) detection and/or measurement means (53, 55, 57) of the ligand-receptor complexes formed during the reaction between the sample and the reagent.

25. A device for detecting the presence of a substance corresponding to one of the two elements of a ligand-receptor pair in an analytical sample, characterised in that it implements a process according to claim 20 and in that it comprises:

a) reception means (47) of two reagents containing respectively the ligand and the receptor, allowing them to be brought into contact in conditions suitable to allow their reaction;

b) means for acquiring an electromagnetic signal from the analytical sample;

c) means (51) for applying the signal delivered by said electronic signal acquisition means (5, 9, 9', 19) to one and/or the other of the reagents; and

d) means (53, 55, 57) for detecting and/or measuring the ligand-receptor complexes formed during the reaction between the two reagents.

26. A device according to claim 24 or claim 25, characterised in that the detection means comprise optical detection means.

27. A device according to any one of claims 24 to 26, characterised in that it includes an enclosure (13) fitted with an electrical and magnetic shielding surrounding said reception means (47).

28. Application of a process for detecting the presence of a substance in analytical sample according to any one of the claims 20 to 23 to biological diagnostics in human or veterinary medicine.

29. Application of a process for detecting the presence of a substance in an analytical sample according

to any one of the claims 20 to 23 to bacteriological control in the pharmaceutical industry, the cosmetic industry, food production and industries.

30. A process for detecting the presence, in an electromagnetic test signal, of an electromagnetic signal characteristic of the biological activity of a substance corresponding to one of the two elements of a ligand-receptor pair, characterised in that it includes the implementation of an amplification process according to any one of claims 1 to 8 and 15 to 19.

31. A detection process according to claim 30, characterised in that the electromagnetic signal is the electromagnetic signal radiated by an electromagnetic radiation source.

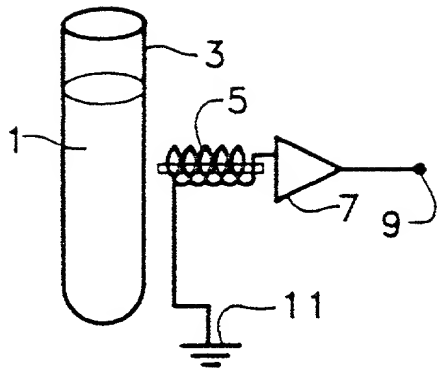


FIG.1

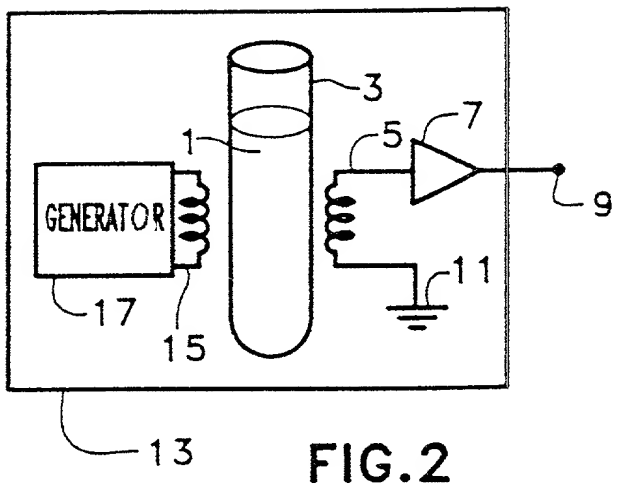


FIG.2

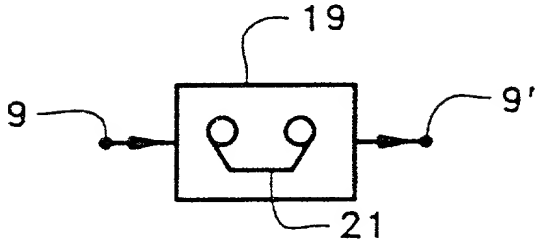


FIG.3

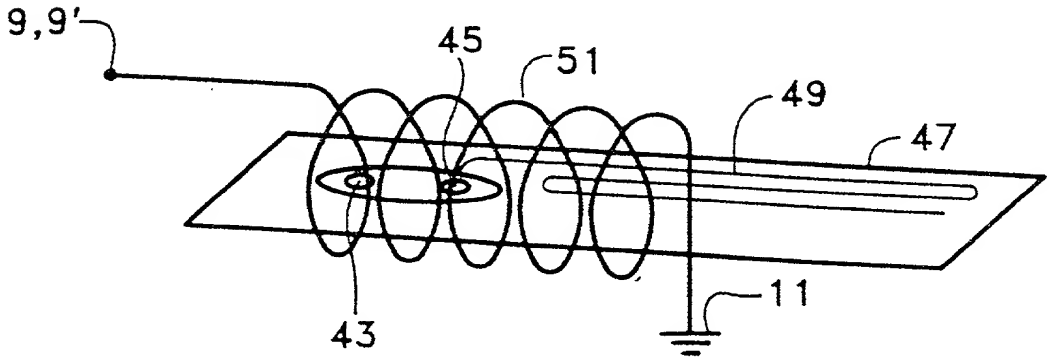


FIG.5

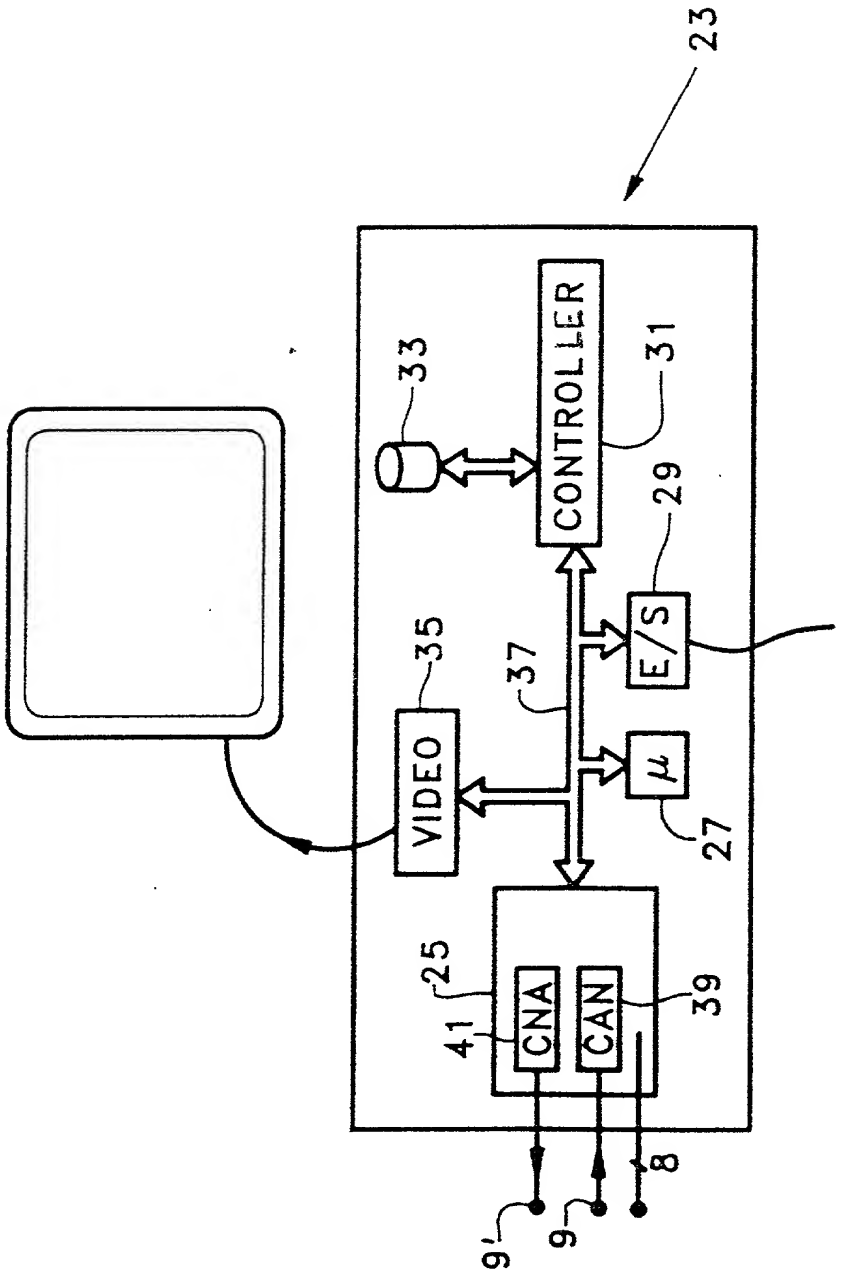


FIG.4

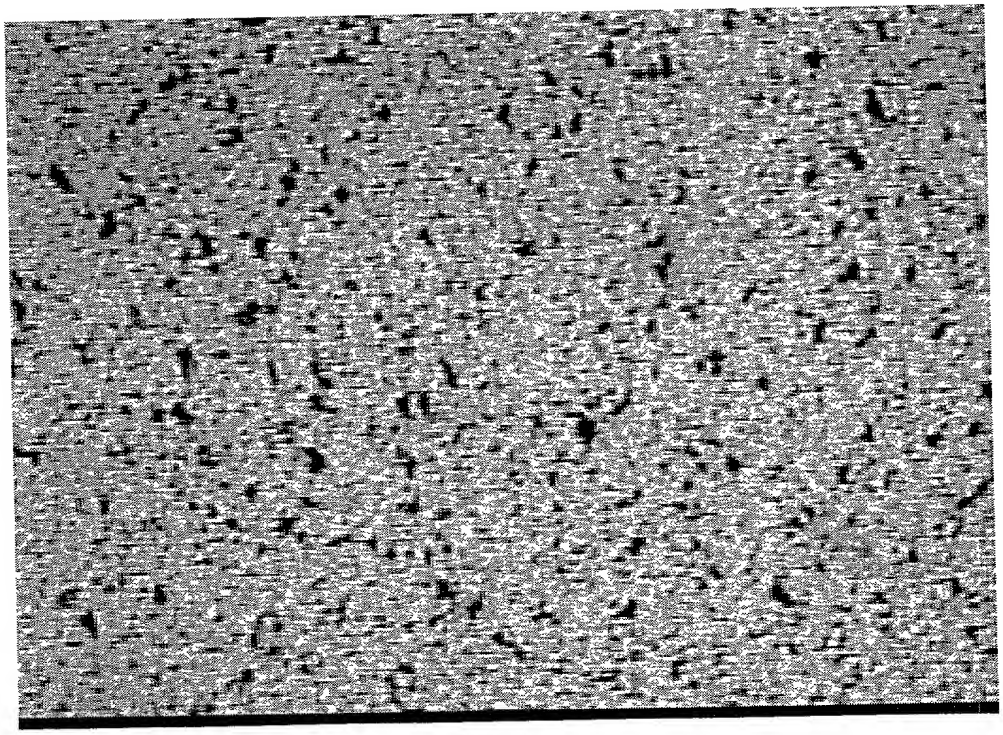


Fig. 6

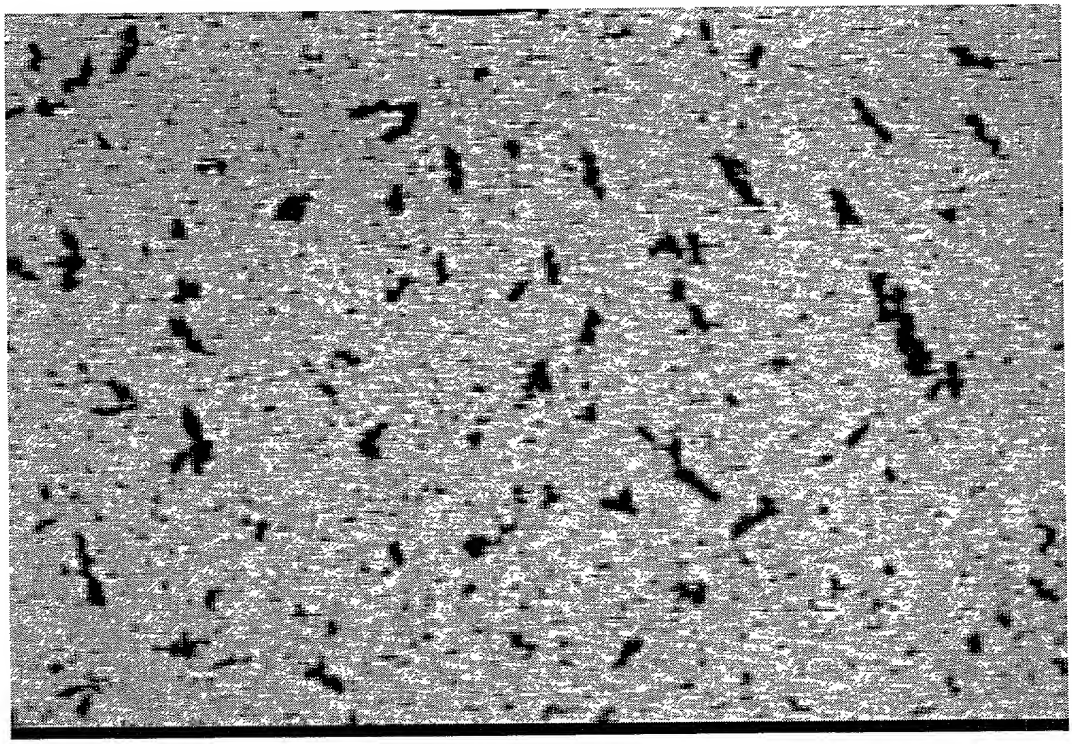


Fig. 7

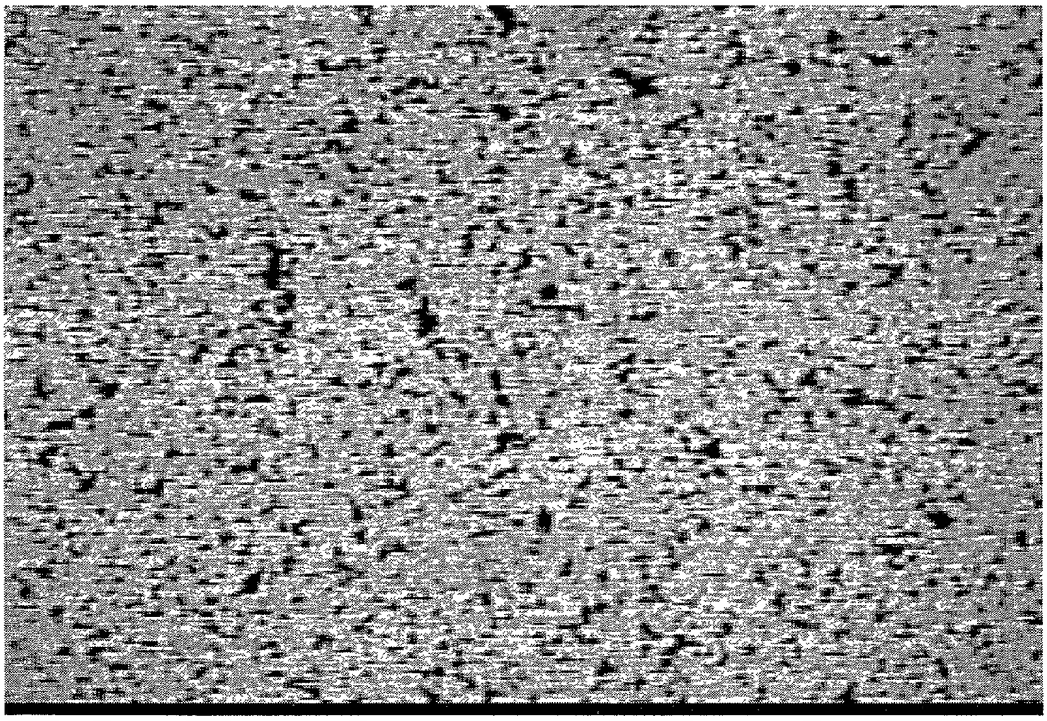


Fig. 8

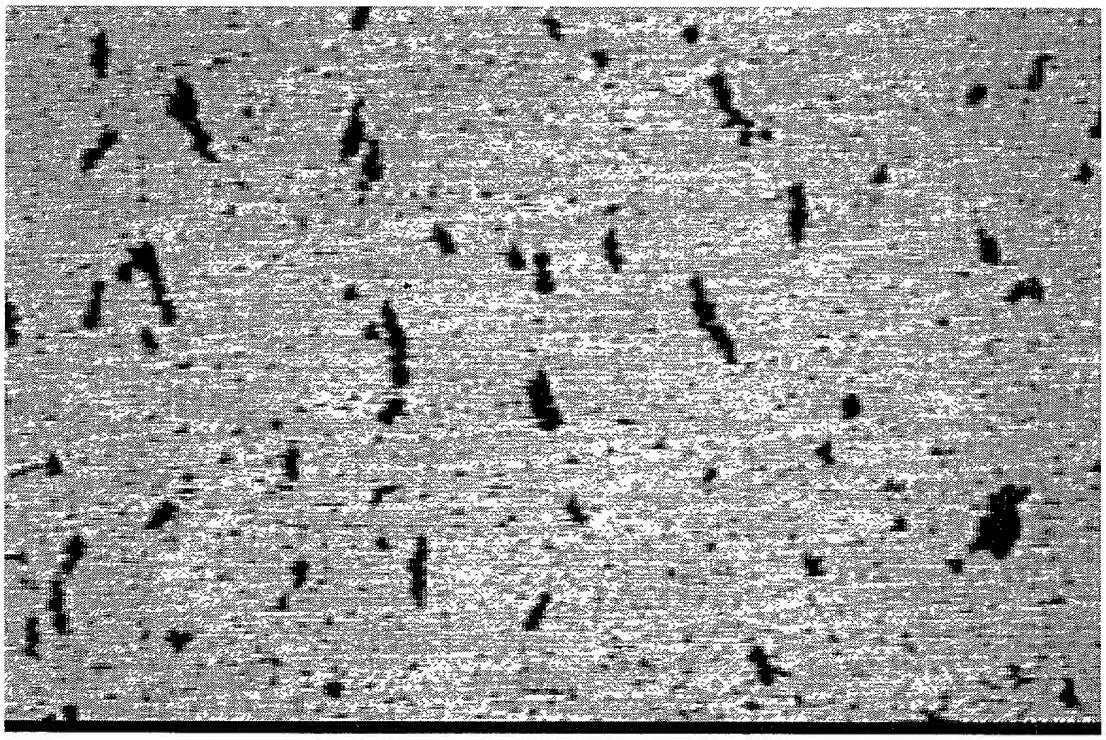


Fig. 9

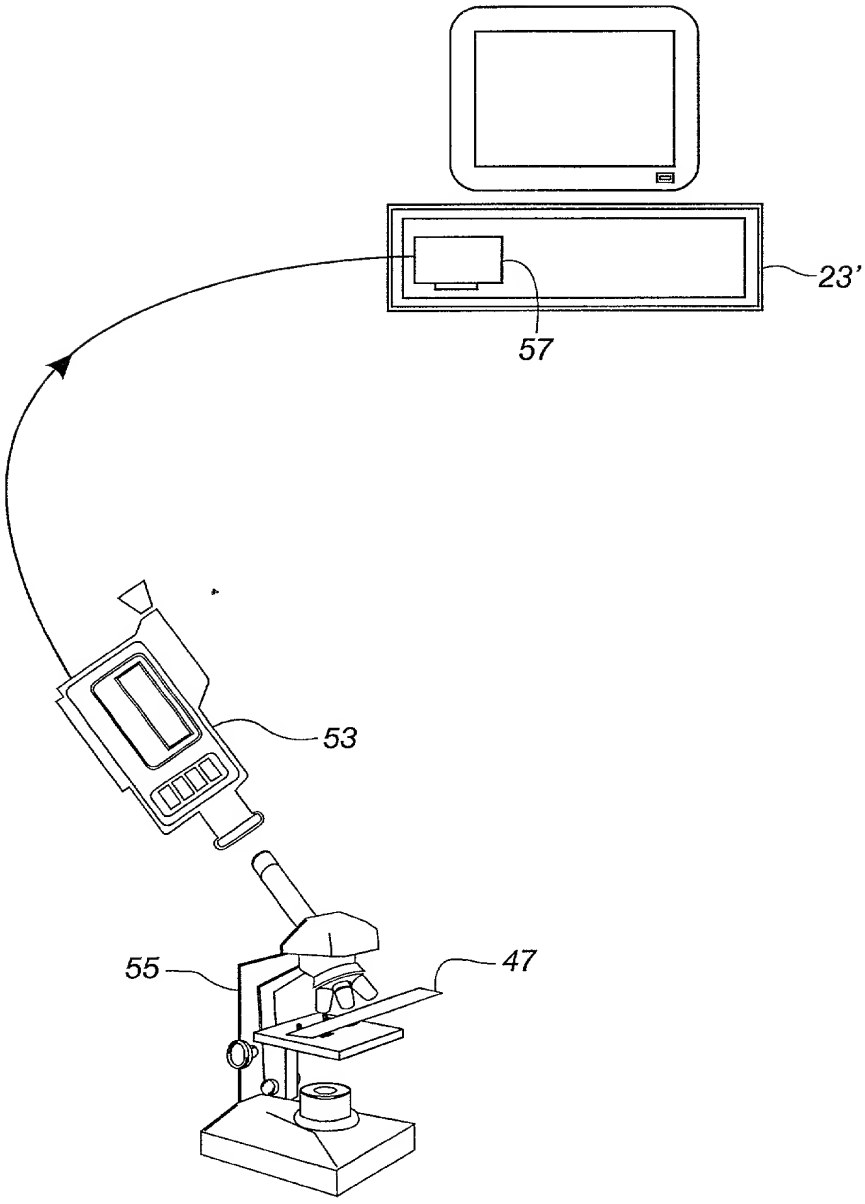


Fig. 10

Attorney Docket No. 9320.113USWO

MERCHANT & GOULD P.C.

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: AMPLIFICATION PROCESS OF THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND ITS USES

The specification of which
a. ☐ is attached hereto
b. ☒ was filed on as application serial no. and was amended on (if applicable) (in the case of a PCT-filed application) described and claimed in international no. PCT/FR99/00915 filed April 19, 1999 and as amended on (if any), which I have reviewed and for which I solicit a United States patent.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (attached hereto).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

a. ☐ no such applications have been filed.
b. ☒ such applications have been filed as follows:

FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC § 119			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**
France	98 04924	20 April 1998	
ALL FOREIGN APPLICATION(S), IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S)			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (day, month, year)**	**DATE OF ISSUE (day, month, year)**

I hereby claim the benefit under Title 35, United States Code, § 120/365 of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. APPLICATION NUMBER	DATE OF FILING (day, month, year)	STATUS (patented, pending, abandoned)

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

U.S. PROVISIONAL APPLICATION NUMBER	DATE OF FILING (Day, Month, Year)

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

Albrecht, John W.	Reg. No. 40,481
Ali, M. Jeffer	Reg. No. 46,359
Anderson, Gregg I.	Reg. No. 28,828
Batzli, Brian H.	Reg. No. 32,960
Beard, John L.	Reg. No. 27,612
Berns, John M.	Reg. No. 43,496
Black, Bruce E.	Reg. No. 41,622
Branch, John W.	Reg. No. 41,633
Bremer, Dennis C.	Reg. No. 40,528
Bruess, Steven C.	Reg. No. 34,130
Byrne, Linda M.	Reg. No. 32,404
Campbell, Keith	Reg. No.P-46,597
Carlson, Alan G.	Reg. No. 25,959
Caspers, Philip P.	Reg. No. 33,227
Chiapetta, James R.	Reg. No. 39,634
Clifford, John A.	Reg. No. 30,247
Coldren, Richard J	Reg. No 44,084
Daignault, Ronald A.	Reg. No. 25,968
Daley, Dennis R.	Reg. No. 34,994
Dalglish, Leslie E.	Reg. No. 40,579
Daulton, Julie R.	Reg. No. 36,414
DeVries Smith, Katherine M.	Reg. No. 42,157
DiPietro, Mark J.	Reg. No. 28,707
Edell, Robert T.	Reg. No. 20,187
Epp Ryan, Sandra	Reg. No. 39,667
Glance, Robert J.	Reg. No. 40,620
Goggin, Matthew J.	Reg. No. 44,125
Golla, Charles E.	Reg. No. 26,896
Gorman, Alan G.	Reg. No. 38,472
Gould, John D.	Reg. No. 18,223
Gregson, Richard	Reg. No. 41,804
Gresens, John J.	Reg. No. 33,112
Hamer, Samuel A.	Reg. No. 46,754
Hamre, Curtis B.	Reg. No. 29,165
Harrison, Kevin C.	Reg. No.P-46,759
Hertzberg, Brett A.	Reg. No. 42,660
Hillson, Randall A.	Reg. No. 31,838
Holzer, Jr., Richard J.	Reg. No. 42,668
Johnston, Scott W.	Reg. No. 39,721
Kadievitch, Natalie D.	Reg. No. 34,196
Karjeker, Shaukat	Reg. No. 34,049
Kastelic, Joseph M.	Reg. No. 37,160
Kettelberger, Denise	Reg. No. 33,924
Keys, Jeramie J.	Reg. No. 42,724
Knearl, Homer L.	Reg. No. 21,197
Kowalchyk, Alan W.	Reg. No. 31,535
Kowalchyk, Katherine M.	Reg. No. 36,848
Lacy, Paul E.	Reg. No. 38,946
Larson, James A.	Reg. No. 40,443
Leon, Andrew J.	Reg. No. 46,869
Leonard, Christopher J.	Reg. No. 41,940
Liepa, Mara E.	Reg. No. 40,066
Lindquist, Timothy A.	Reg. No. 40,701
Lycke, Lawrence E.	Reg. No. 38,540
McAuley, Steven A.	Reg. No. 46,084
McDonald, Daniel W.	Reg. No. 32,044
McIntyre, Jr., William F.	Reg. No. 44,921
Mitchem, M. Todd	Reg. No. 40,731
Mueller, Douglas P.	Reg. No. 30,300
Nichols, A. Shane	Reg. No. 43,836
Pauly, Daniel M.	Reg. No. 40,123
Phillips, Bryan K.	Reg. No. P-46,990
Phillips, John B.	Reg. No. 37,206
Plunkett, Theodore	Reg. No. 37,209
Prendergast, Paul	Reg. No. 46,068
Pytel, Melissa J.	Reg. No. 41,512
Qualey, Terry	Reg. No. 25,148
Reich, John C.	Reg. No. 37,703
Reiland, Earl D.	Reg. No. 25,767
Samuels, Lisa A.	Reg. No. 43,080
Schmaltz, David G.	Reg. No. 39,828
Schuman, Mark D.	Reg. No. 31,197
Schumann, Michael D.	Reg. No. 30,422
Scull, Timothy B.	Reg. No. 42,137
Sebald, Gregory A.	Reg. No. 33,280
Skoog, Mark T.	Reg. No. 40,178
Spellman, Steven J.	Reg. No. 45,124
Stoll-DeBell, Kirstin L.	Reg. No. 43,164
Sumner, John P.	Reg. No. 29,114
Swenson, Erik G.	Reg. No. 45,147
Tellekson, David K.	Reg. No. 32,314
Trembath, Jon R.	Reg. No. 38,344
Tuchman, Ido	Reg. No. 45,924
Underhill, Albert L.	Reg. No. 27,403
Vandenburgh, J. Derek	Reg. No. 32,179
Wahl, John R.	Reg. No. 33,044
Weaver, Karrie G.	Reg. No. 43,245
Welter, Paul A.	Reg. No. 20,890
Whipps, Brian	Reg. No. 43,261
Whitaker, John E.	Reg. No. 42,222
Wickhem, J. Scot	Reg. No. 41,376
Williams, Douglas J.	Reg. No. 27,054
Withers, James D.	Reg. No. 40,376
Witt, Jonelle	Reg. No. 41,980
Wu, Tong	Reg. No. 43,361
Xu, Min S.	Reg. No. 39,536
Zeuli, Anthony R.	Reg. No. 45,255

I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Merchant & Gould P.C. to the contrary.

Please direct all correspondence in this case to Merchant & Gould P.C. at the address indicated below:

Merchant & Gould P.C.
P.O. Box 2903
Minneapolis, MN 55402-0903

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2	**Full Name Of Inventor**	**Family Name** Benveniste	**First Given Name** Jacques	**Second Given Name**
0	**Residence & Citizenship**	**City** Paris	**State or Foreign Country** France FRX	**Country of Citizenship** France
1	**Post Office Address**	**Post Office Address** 3, rue Larochelle	**City** Paris	**State & Zip Code/Country** 75014 / France
Signature of Inventor 201:			**Date:** Nov. 8, 2000	

2	**Full Name Of Inventor**	**Family Name** Guillonnet	**First Given Name** Didier	**Second Given Name**
0	**Residence & Citizenship**	**City** Cagnes-sur-mer	**State or Foreign Country** France FRX	**Country of Citizenship** France
2	**Post Office Address**	**Post Office Address** 121, Chemin du Val de Cagnes	**City** Cagnes-sur-mer	**State & Zip Code/Country** 06800 / France
Signature of Inventor 202:			**Date:** Nov 8, 2000	

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application:

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division

SCANNED, # 6

Application deficiencies found during scanning:

☐ Page(s)_____of_____ were not present for scanning. (Document title)

☐ Page(s)_____of_____were not present for scanning. (Document title)

There is no pages Between 34 – 55

☐ ***Scanned copy is best available.***